IL-13 Protects Mice from Lipopolysaccharide-Induced Lethal Endotoxemia

Correlation with Down-Modulation of TNF-α, IFN-γ, and IL-12 Production[1]

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IL-13 is a potent down-modulator of macrophage proinflammatory activity in vitro, similar in this context to the anti-inflammatory cytokines IL-4 and IL-10. Since IL-10 effectively confers protection to mice from LPS-induced lethal endotoxemia through inhibition of proinflammatory cytokine production, we investigated whether IL-13 may also be capable of providing protection in this experimental model of endotoxic shock. A single injection of recombinant murine IL-13 (rmIL-13; 0.5–10 μg) significantly increased survival in a dose-dependent manner when a lethal i.p. injection of endotoxin was administered to BALB/c mice. This effect appeared to be IL-13 specific, since survival was not affected in mice that received heat-inactivated rmIL-13. rmIL-13 provided significant protection to mice even when given 30 min after LPS injection; however, this protection decreased in a time-dependent manner as the administration of rmIL-13 was delayed by 1, 2, and 5 h following LPS injection. The protective effect of IL-13 was correlated with significant decreases in the production of the inflammatory mediators TNF-α, IFN-γ, and IL-12 as well as a decrease in the anti-inflammatory mediator IL-10. Our data suggest that IL-13 provides protection from LPS-induced lethal endotoxemia in a manner that is similar to but independent from that of IL-10, and therefore can be added to the list of cytokine immunomodulators that might be beneficial in the treatment of septic shock. *The Journal of Immunology,* **1997, 158: 2898–2903.**

Severe Gram-negative bacterial infections in humans can result in endotoxic shock, a pathophysiologic state characterized by hypotension, multiple organ dysfunction, and potentially death. The LPS (endotoxin) component of the bacterial cell wall is the main causative agent of this toxicity (1, 2). Indeed, experimental models of endotoxic shock have demonstrated that a single injection of LPS into animals can produce changes that are characteristic of the septic shock syndrome in humans. Endotoxin exerts its effect by inducing potent macrophage activation, with the sequential release of proinflammatory cytokines such as TNF-α, IL-12, IL-1, IL-6, and IL-8 (3). IL-12, in concert with TNF-α or B7 costimulation, can further act as a potent inducer of IFN-γ production by T and NK cells (4–6). The central importance of these cytokines in the pathogenesis of endotoxic shock is suggested by the fact that high circulating levels of these cytokines can be found in the serum of both humans and animals during endotoxemia, and that administration of neutralizing anti-cytokine Abs or IL-1Ra,[3] or gene targeting of cytokine receptors such as the p55TNFR or IFN-γR, can either greatly diminish or completely abrogate the lethality associated with endotoxemia in a variety of animal models (3, 7–17).

Substantial evidence from both in vitro and in vivo studies suggests that the response of hemopoietic cells to LPS involves not only the rapid up-regulation of proinflammatory cytokines, but the concomitant induction of potent anti-inflammatory cytokines such as IL-10 and IL-1Ra (3, 18). In vitro studies have shown that IL-10 is a potent monocyte/macrophage-deactivating cytokine capable of suppressing the induced production of proinflammatory cytokines, while at the same time up-regulating production of IL-1Ra (19–22). IL-10 is also a potent inhibitor of in vitro IL-12 production by monocytes/macrophages, and this at least partially accounts for the ability of IL-10 to act as a potent inhibitor of IFN-γ production by T and NK cells (4–6). In vivo experiments in mice have confirmed and extended these findings, showing that neutralization of IL-10 activity with a specific mAb or elimination of IL-10 production by means of targeted disruption of the IL-10 gene leads to elevated levels of serum TNF-α and IFN-γ (23–27). Furthermore, both IL-10-deficient mice and anti-IL-10-pretreated mice manifest enhanced endotoxin sensitivity, which correlates with the observed increase in serum levels of both TNF-α and IFN-γ (24, 26, 27). Consistent with these findings, IL-10 has been shown to act as a potent immunomodulator of cytokine production and lethality in animal models of LPS-induced lethal endotoxemia (26–29).

IL-13 is another cytokine, produced by T cells and mast cells, that potently down-modulates the production of proinflammatory cytokines by LPS-activated monocytes, while at the same time enhancing the secretion of IL-1Ra (30–36). The fact that IL-13 strongly suppresses cytokine production by activated monocytes even in the presence of anti-IL-10 or anti-IL-4 Abs (32, 33) and the fact that IL-13 directly inhibits macrophage IL-10 production (32) indicate that these cytokines act independently to effect suppression of macrophage activity. This point is underscored by the fact that while IL-10 is considered a general deactivator of macrophage function, suppressing macrophage MHC class II Ag expression

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Received for publication March 12, 1996. Accepted for publication December 10, 1996.

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[3] Abbreviations used in this paper: IL-1Ra, interleukin-1 receptor agonist; LD_{90}, lethal dose 90, dose at which 90% mortality occurs; rm, recombinant murine; HPRT, hypoxanthine phosphoribosyltransferase.

0022-1767/97/$02.00

and APC function, IL-13 does not act as a general monocyte deactivator. In contrast, IL-13 up-regulates MHC class II Ag expression on macrophages and has little effect on APC function (32, 33). IL-13 can also enhance human B cell proliferation, up-regulate the expression of the MHC class II Ag and other B cell surface Ags, and act as a switch factor for induction of germline ϵ transcription and IgE production (30, 37–40). The ability of IL-13 to act as a potent down-modulator of macrophage proinflammatory cytokine production while at the same time preserving macrophage APC activity and B cell effector function makes IL-13 an attractive candidate for therapeutic intervention in septic shock. We test here, in an experimental model of endotoxic shock, whether IL-13 is able to confer protection against LPS-induced lethal endotoxemia, and whether, like IL-10, this protection is correlated with down-modulation of a proinflammatory cytokine response.

Materials and Methods

Mice

Eight-week-old female BALB/c mice were purchased from Harlan Sprague-Dawley (Pratville, AL) and kept at the DNAX Research Institute animal facility (Palo Alto, CA) for 2.5 days before experimentation to minimize experimental variation caused by inconsistent animal management.

Reagents

LPS from *Escherichia coli* (serotype 0111:B4) was obtained from Difco Laboratories (Detroit, MI). The protein concentration of the LPS preparation was determined to be approximately 50 μg protein/10 mg/ml LPS (~5%). Recombinant murine (rm) was expressed in *E. coli* and purified as previously described (28). rmIL-13 was generated as follows. The coding region for mIL-13 was subcloned into the pFLAG vector (Kodak Co., New Haven, CT) and transfected into the Topp 3 strain of *E. coli*. Cultures were grown at 37°C in Luria broth media until the OD_{560} reached 0.7. The cultures were then transferred to 20°C and allowed to equilibrate, after which protein expression was induced by the addition of isopropyl-β-D-thiogalactopyranoside to a final concentration of 0.4 mM. After growth overnight, the cells were harvested by centrifugation, and the FLAG rmIL-13 was extracted from the cell pellet by using a modified osmotic shock procedure using sucrose or NaCl (41). The osmotic extract was loaded onto a 25-ml column of M2 affinity resin (IBI, New Haven, CT), the column was washed with PBS, and the specifically bound material was eluted with 0.1 M glycine, pH 3.0. Peak fractions from the M2 column were pooled and chromatographed over a Poros Q column (PerSeptive Biosystems, Inc., Cambridge, MA) at pH 7.5 and 5.0 ms. The FLAG rmIL-13 was present in the flow-through and wash fractions. These fractions were further purified over a Poros reverse phase column (PerSeptive Biosystems), using a linear gradient of acetonitrile from 0 to 80% containing 0.1% trifluoroacetic acid to elute the bound protein. Peak fractions from the Poros reverse phase column were pooled and dialyzed into FLAG cleavage buffer (Biozyme Laboratories, San Diego, CA). The FLAG peptide was cleaved with enterokinase (Biozyme Laboratories; 1000 U/mg of fusion protein), and the rmIL-13 was separated from the peptide by reverse phase chromatography. The fractions were dialyzed into 50 mM sodium acetate, pH 5.5, and stored at −70°C. The purity of the protein was determined by analysis of Coomassie-stained 10% polyacrylamide gels (Tricine, Novex, San Diego, CA), and the concentration was determined by scanning the gels on a densitometer (Molecular Dynamics Corp., Sunnyvale, CA). Endotoxin levels of rmIL-13 preparations ranged from 10.0 to 330.0 Eu/ml (10 Eu = 1 ng).

Experimentally induced endotoxic shock

Mice were injected i.p. with a predetermined LD_{90} (100 μg) of LPS, and survival was monitored over the next 7 days. Various doses of rmIL-13 were administered i.p. either concurrently with or following injection of LPS. rmIL-10 (5 μg) was administered i.p. concurrently with LPS to serve as a positive control for survival. Inactivation of rmIL-13 biologic activity was achieved by adjusting the buffer from pH 5.5 to 7.0, followed by autoclaving. Inactivation of rmIL-13 activity was confirmed using a bioassay based on proliferation of the human premyeloid cell line TF-1 (30). All survival studies were conducted in a blinded and randomized fashion.

Measurement of cytokine levels by ELISA assay

Mice receiving LPS concurrently with either PBS or rmIL-13 were anesthetized with CO_2, and serum was collected at various time points through cardiac puncture. Serum TNF-α levels were measured using a commercially available murine-specific sandwich ELISA (Genzyme, Cambridge, MA). Serum IFN-γ and IL-12 p40 levels were measured using modified two-site sandwich ELISA assays (42). The mAbs used for detection of IFN-γ were R46A2 and AN18 (42) and were kindly provided by Dr. John Abrams (DNAX). The mAbs used for detection of the p40 subunit of mIL-12 were C15.1.2 and C15.6.7 (43) and were kindly provided to DNAX by Dr. Giorgio Trinchieri.

Measurement of RNA by reverse transcriptase-PCR analysis

BALB/c mice were injected i.p. with LPS alone, LPS in combination with rmIL-13, or rmIL-13 alone, and total RNA was extracted from the pooled spleens of three mice by the RNAzol B method (Tel-Test, Inc., Friendswood, TX). Equal amounts of total RNA (1 μg/sample) were reverse transcribed essentially as described in the GeneAmp RNA PCR kit (Perkin-Elmer Corp., Norwalk, CT) using random hexamer primers and Superscript RNase H^- reverse transcriptase (Life Technologies, Gaithersburg, MD) in a final reaction volume of 20 μl. This reaction was scaled up so that several DNA amplifications could be set up from the same reverse transcriptase reaction per given RNA sample. Reactions were preincubated for 10 min at room temperature, and reverse transcribed for 15 min at 42°C in a DNA thermal cycler. Samples were placed on ice. RNase H (Life Technologies; 1 μl/20-μl sample) was added to each sample, and the reactions were incubated at 37°C for 30 min. Twenty microliters of the reverse transcriptase reaction was amplified in a final volume of 50 μl using sequence-specific primers at a final concentration of 1 μM, and AmpliTaq DNA polymerase (Perkin-Elmer Corp.) at 2.5 U/reaction. The primers used were mIL-12 p40 (5′ primer, atgtgtcctcagaagctaaccatc; 3′ primer, cactgtaggag gaccgtcctgtga), mIL-12 p35 (5′ primer, atgtgtcaatcacgctgtaagtac; 3′ primer, aggcgtgaagcaggatgcagagcttc), mIL-10 (5′ primer, ccttaatgcaggactt taagg; 3′ primer, gacaccttggtcttggagc), and mHPRT (5′ primer, gtaatgat cagtcaacgggggac; 3′ primer, ccagcaagcttgcaaccttaacca). All primer sequences are written in the 5′ to 3′ direction. The sizes of the resulting DNA amplification products are: mIL-12 p40, 586 bp; mIL-12 p35, 591 bp; mIL-10, 240 bp; and HPRT, 214 bp. All PCR amplification reactions were conducted as 20 cycles of 5-min denaturation at 95°C, 2-min annealing at 58°C, and 3-min extension at 72°C. These conditions were predetermined such that reverse transcriptase-PCR analysis of serial dilutions of individual RNA samples (0.1–2.5 μg of RNA) resulted in a linear signal detection with the primer sets chosen (data not shown). Samples were electrophoresed (one-fifth of the PCR reaction) on 1% agarose gels, followed by transfer to Nytran nylon membranes (Schleicher and Schuell, Keene, NH). Murine IL-12 p40, IL-12 p35, IL-10, and HPRT DNA amplification products were detected by hybridization to ^{32}P-labeled cDNA probes. The filters were exposed to X-OMAT AR film (Eastman Kodak Co., Rochester, NY).

Statistical analysis

Survival curves (Fig. 1) were analyzed by the Kaplan-Meier method, and Wilcoxon statistics were generated to test the homogeneity between treatment groups. Delayed cytokine administration data (Fig. 3) were analyzed for statistical significance using Dunnett's method, a post-hoc parametric means comparison of experimental treatments compared with a control. Serum cytokine levels (Fig. 4) in different experimental groups were analyzed for statistical significance using the nonparametric Wilcoxon rank sum test. All statistical analyses were performed using JMP software (SAS Institute, Cary, NC).

Results and Discussion

In the present experiments we have used a well-established model of endotoxic shock to determine whether IL-13, a novel cytokine with potent anti-inflammatory properties, is capable of exerting immunomodulatory effects on LPS-induced lethal endotoxemia. Groups of 20 mice were challenged i.p. with 100 μg of LPS, a dose predetermined to be lethal in 80 to 90% of the animals, in combination with either PBS or various doses of rmIL-13. In five identical independent experiments, a single injection of 10, 5, 1, or 0.5 μg of rmIL-13 reproducibly and significantly ($p < 0.01$) protected mice from LPS-induced lethality in a dose-dependent manner, with

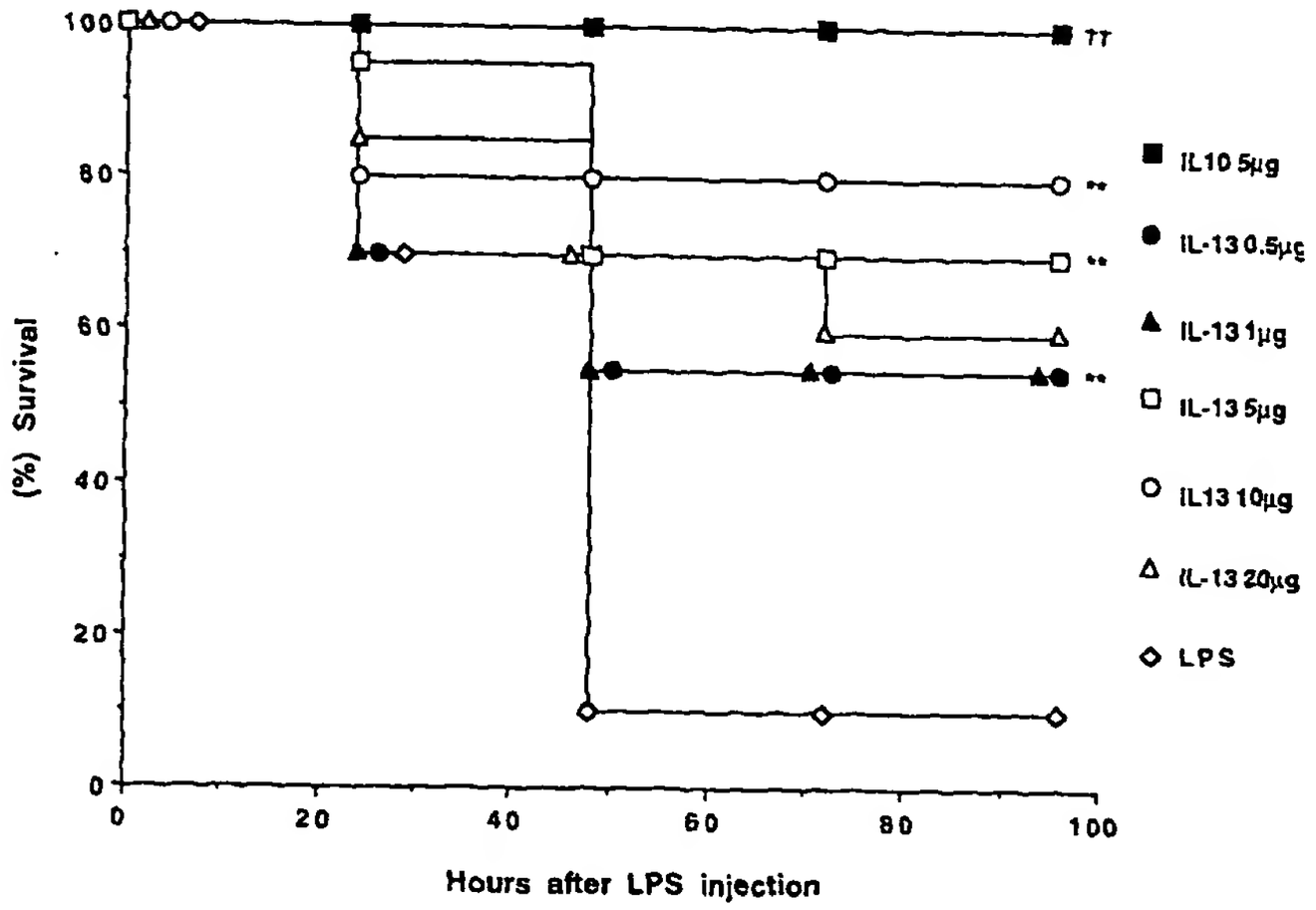

FIGURE 1. rmIL-13 protects mice from LPS-induced lethality. Groups of 20 BALB/c mice were injected i.p. with 100 μg of LPS, and either PBS (◇) or PBS containing 0.5 μg (●), 1 μg (▲), 5 μg (■), 10 μg (○), or 20 μg (△) of rmIL-13. Another group of mice received 100 μg of LPS together with 5 μg of rmIL-10 (■) to serve as a positive control for 100% protection from LPS-induced lethality. Survival was monitored over the next 7 days. Similar results were obtained in five identical independent experiments. The survival curves from all five experiments were analyzed by the Kaplan-Meier method, and Wilcoxon statistics were generated to test the homogeneity between treatment groups (LPS plus rmIL-13 vs an LD_{90} of LPS alone (** indicates $p < 0.01$); LPS plus rmIL-10 vs LPS plus rmIL-13 (†† indicates $p < 0.01$)).

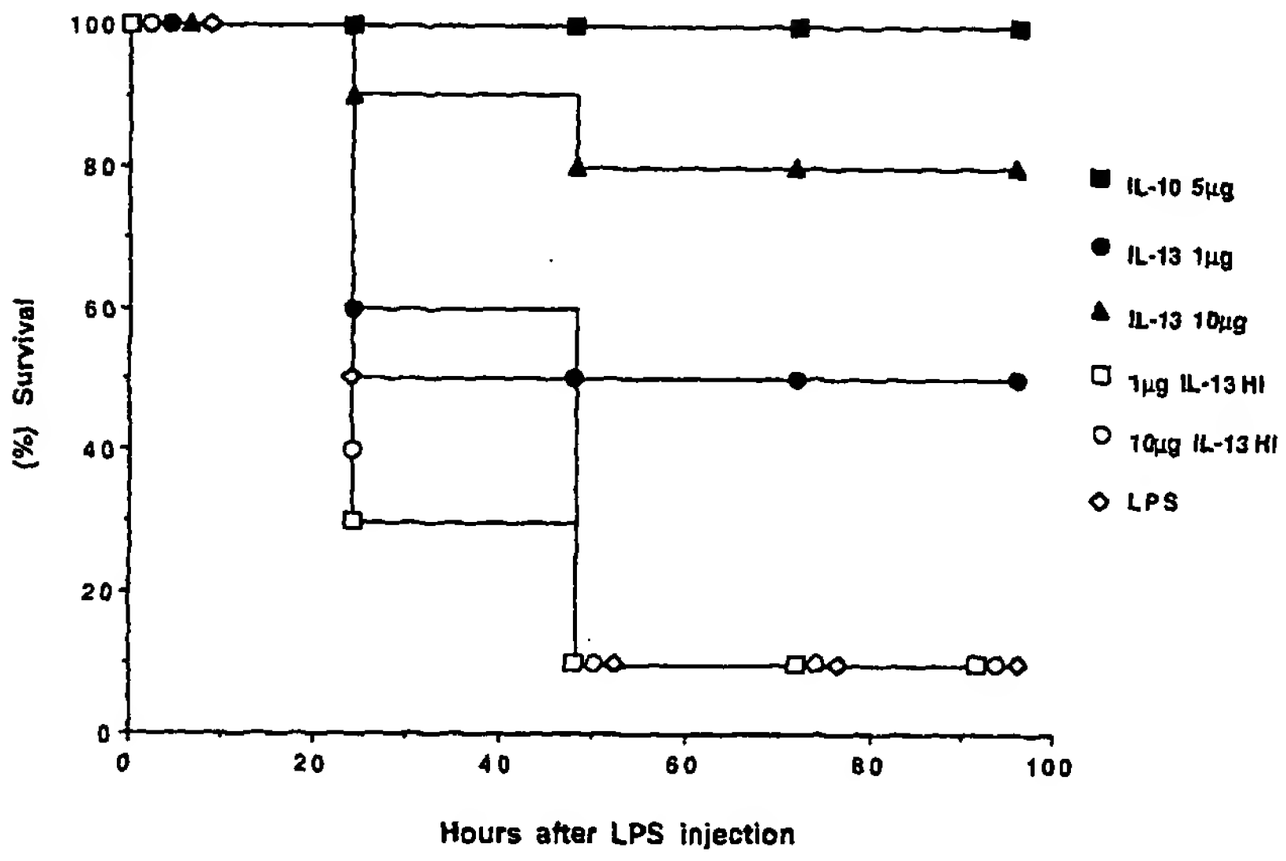

FIGURE 2. Administration of heat-inactivated rmIL-13 does not affect LPS-induced lethality. Groups of 10 BALB/c mice were injected i.p. with 100 μg of LPS and PBS (◇), PBS containing 1 μg (●) or 10 μg (▲) of rmIL-13, or PBS containing 1 μg (□) or 10 μg (○) of heat-inactivated rmIL-13. Another group of mice received 100 μg of LPS together with 5 μg of rmIL-10 to serve as a positive control for 100% protection from LPS-induced lethality. Survival was monitored over the next 7 days. Similar results were obtained in three identical independent experiments.

10 μg of rmIL-13 protecting 80% of the mice (representative experiment shown in Fig. 1). In some experiments a slight decrease in survival was observed when mice received 20 μg (Fig. 1) or 40 μg (data not shown) of rmIL-13, suggesting that IL-13 may be toxic at higher concentrations. Consistent with previous findings by us and others, concurrent administration of 5 μg of rmIL-10 and 100 μg of LPS routinely resulted in 100% protection from LPS-induced lethality (Figs. 1 and 2). Analysis of survival curves (Fig. 1) confirmed that at the dose administered, rmIL-10 was significantly more effective ($p < 0.01$) than any dose of rmIL-13 tested at protecting mice from LPS-induced shock. LPS-treated animals injected with suboptimal doses of rmIL-10 (0.05 μg) and varying doses of rmIL-13 (0.1–10.0 μg) showed no evidence of a synergistic protective response (data not shown). The specificity of the IL-13-mediated protection against LPS-induced shock could not be evaluated using neutralizing anti-IL-13 mAbs, since no such Abs have yet been produced. As an alternative approach to determining whether protection from LPS-induced lethality was specifically due to the administration of rmIL-13, the rmIL-13 preparation was heat inactivated and tested for protective activity. The loss of biologic activity following heat inactivation was confirmed using the factor-dependent premyeloid cell line TF-1 (data not shown). Administration of 1 or 10 μg of heat-inactivated rmIL-13 at the time of LPS injection did not result in protection from LPS-induced lethality (Fig. 2), supporting the specificity of this effect. A kinetics study revealed that rmIL-13 significantly ($\alpha = 0.05$) protected mice even when given 30 min after LPS injection (Fig. 3); however, survival decreased substantially as the administration of rmIL-13 was delayed to either 1 or 2 h, and protection was virtually absent by 5 h after LPS injection. Taken together, these data indicate that IL-13 is highly effective at conferring protection to mice from the lethality associated with LPS-induced endotoxemia when administered either together with, or up to 30 min following induction of shock. IL-13 is not as effective as IL-10, however, which confers 100% protection to mice when administered at

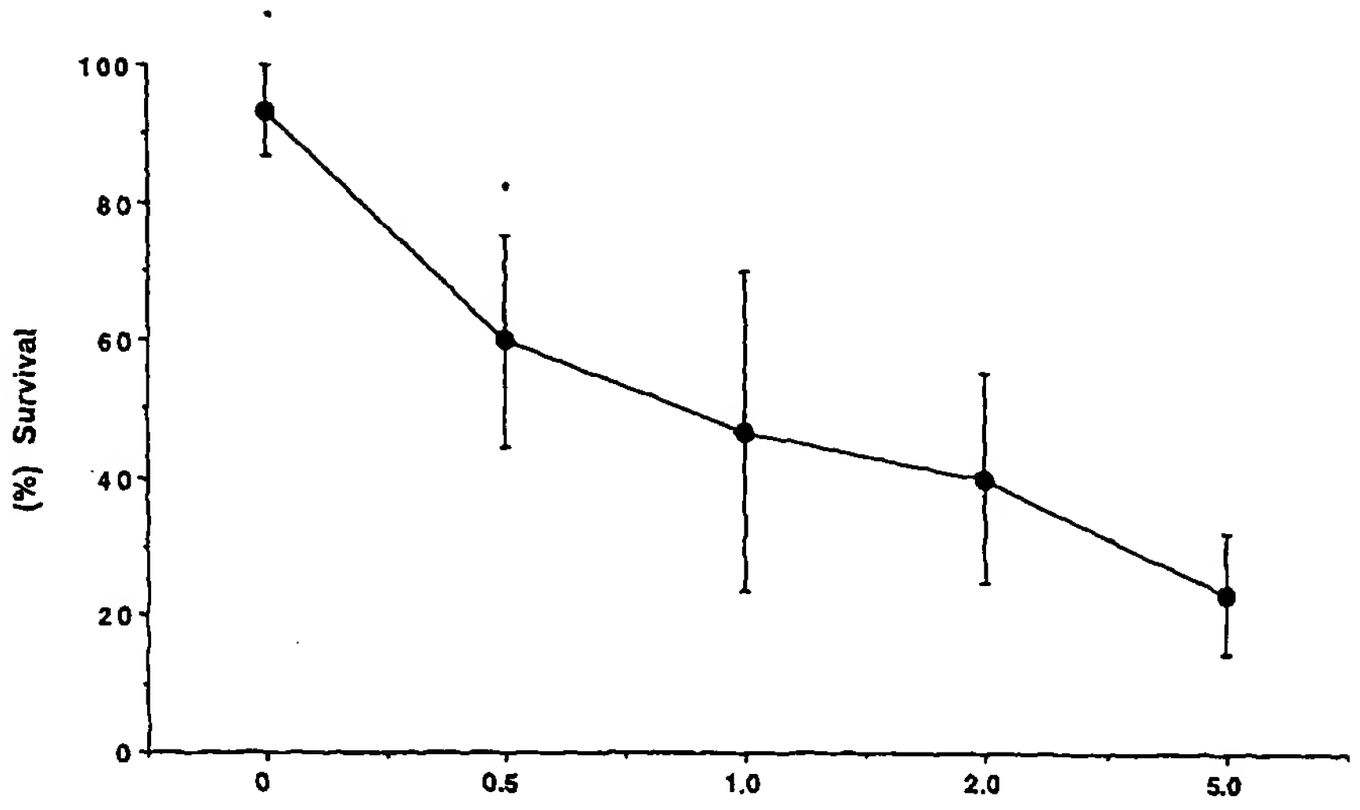

FIGURE 3. rmIL-13 protects mice when administered after LPS injection. Groups of 10 BALB/c mice were injected i.p. with 100 μg of LPS (LD_{90}), and either PBS or PBS containing 10 μg of rmIL-13 (●) was administered 0, 0.5, 1, 2, or 5 h after LPS injection. Survival was monitored over the next 7 days. Survival is represented as the mean ± SEM of three identical independent experiments. * denotes the mean percent surviving values that were significantly different ($\alpha = 0.05$) from the LPS plus PBS control group as determined by the Dunnett's method, comparing multiple means against a control.

time zero and significant protection against lethality when administered up to 1 h following LPS-induced shock (28).

TNF-α, IL-1, IL-6, IFN-γ, and more recently IL-12 have been shown to play pivotal roles in LPS-induced endotoxic shock (3, 7, 8, 18). Indeed, inhibition of the LPS-induced cascade of proinflammatory cytokines is the primary mechanism through which anti-inflammatory immunomodulators such as IL-10 confer protection against the lethal effects of LPS administration (26–29). On the based of the ability of IL-13 to down-modulate cytokine production by LPS-activated monocytes/macrophages, we reasoned that the in vivo protective effect of rmIL-13 on LPS-induced lethal endotoxemia might be mediated through a similar mechanism. To evaluate the effects of rmIL-13 administration on TNF-α, IFN-γ, and IL-12 production during lethal endotoxemia, two groups of 90 mice were injected with an LD_{90} of LPS concurrently with either PBS or 10 μg of rmIL-13, and serum was collected from 10 mice per time point at 0, 1, 2, 3, 4, 5, 7, 9, and 11 h. Consistent with previous findings (15, 26, 28), serum TNF-α levels peaked by 1 and 2 h after LPS injection and rapidly declined thereafter (Fig. 4*A*). In mice that received rmIL-13 in combination with LPS, serum TNF-α levels were significantly reduced at 1, 2, and 3 h ($p < 0.001$), showing little increase over background serum levels (Fig. 4*A*). Also consistent with previous findings (15, 26), serum IFN-γ levels peaked 7 to 9 h following LPS administration, and levels were still relatively high at 11 h (Fig. 4*B*). In contrast, mice that received rmIL-13 in combination with LPS showed a significant reduction of serum IFN-γ levels at 5, 7, 9, and 11 ($p < 0.001$) h, with IFN-γ levels almost at background by 11 h (Fig. 4*B*). To assess the effects of rmIL-13 on IL-12 production during LPS-induced endotoxemia, serum from the above experiment was analyzed for the presence of IL-12 using an ELISA that detects the p40 chain of IL-12 either in free form or associated with the p35 chain in the biologically active p70 heterodimer. Consistent with previous observations (7, 8), increased levels of p40 were detected in the serum of mice between 3 and 7 h after LPS injection, and this preceded the appearance of IFN-γ in the serum (Fig. 4*C*). When rmIL-13 was administered in conjunction with LPS, there was a significant inhibition of LPS-induced IL-12 p40 production at 3, 5, 7, 9, and 11 h ($p < 0.001$; Fig. 4*C*).

The effect of rmIL-13 on LPS-induced IL-12 production during lethal endotoxemia was further examined by reverse transcriptase-PCR analysis. Mice were challenged with 100 μg of LPS concurrently with either PBS or 10 μg of rmIL-13, or with 10 μg of rmIL-13 alone, and total RNA was prepared from the pooled spleens of three mice sacrificed at 0, 1, 3, 5, 7, 9, and 11 h. The RNA was analyzed for steady state levels of mRNA transcripts corresponding to the p35 and p40 subunits of mIL-12. Consistent with previous observations (4, 7, 44), LPS induced the rapid accumulation of p40 mRNA transcripts, with peak accumulation above basal levels occurring between 1 and 7 h after LPS administration (Fig. 5). Administration of rmIL-13 in combination with LPS resulted in a slight increase in p40 mRNA levels at 1 h; however, beginning 3 h after LPS injection, rmIL-13 resulted in a marked inhibition of IL-12 p40 mRNA transcripts (Fig. 5). Treatment with IL-13 alone did not modulate the production of IL-12 p40 mRNA transcripts over basal levels. Also consistent with previous observations, mRNA transcripts corresponding to the p35 subunit of IL-12 were constitutively expressed, and these levels were not significantly altered by the in vivo administration of LPS (4, 7, 44), rmIL-13, or rmIL-13 in combination with LPS (data not shown). HPRT mRNA transcripts were also measured in this experiment, and neither LPS nor rmIL-13, alone or in combination, altered the level of these transcripts following in vivo stimulation (Fig. 5). Taken together, these data are consistent with previous in vitro observations that IL-13 can markedly inhibit LPS-induced IL-12 production by human PBMC, and murine bone marrow-derived macrophages at the level of both cytokine production and accumulation of IL-12 mRNA transcripts (32, 33, 45). In addition, these data extend these observations by demonstrating that IL-13 down-modulates IL-12 production in vivo during LPS-induced lethal endotoxemia.

The induction of endogenous IL-10 is believed to play an important role in modulating proinflammatory cytokine production by LPS-activated monocytes/macrophages (19, 20), as well as during LPS-induced endotoxemia (18, 26, 27). Since IL-13 has been shown to directly inhibit macrophage IL-10 production in vitro (32), we wanted to determine whether IL-13 could mediate a similar down-modulation of endogenous IL-10 production in vivo following LPS administration. The effect of rmIL-13 on the induction of endogenous IL-10 following LPS injection was measured in the reverse transcriptase-PCR experiment described above. Consistent with previous observations (26, 27), LPS administration resulted in an up-regulation of IL-10, with our data demonstrating that IL-10 mRNA transcripts increased in a time-dependent manner from 1 to

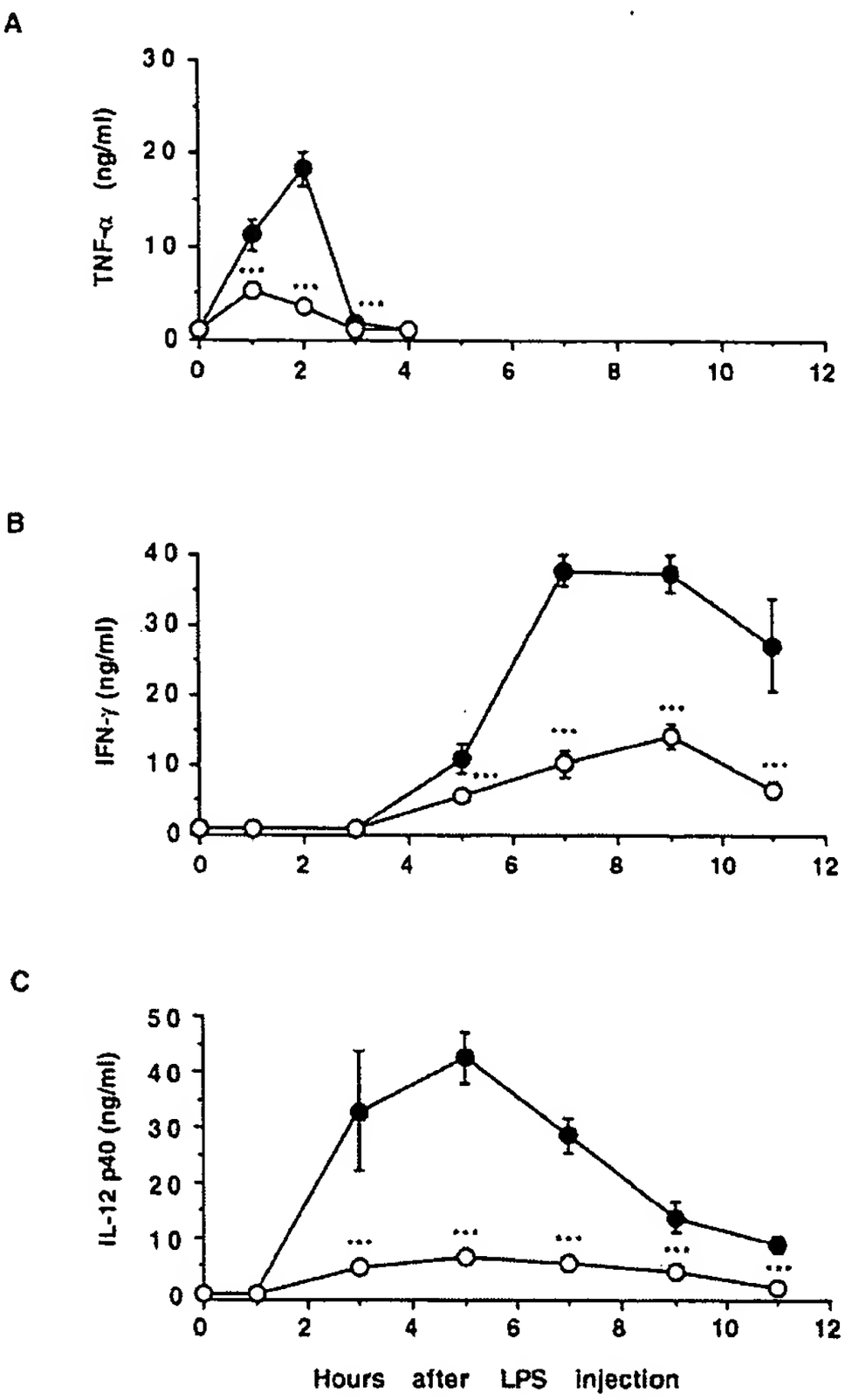

FIGURE 4. rmIL-13 inhibits the production of TNF-α, IFN-γ, and IL-12 p40 during LPS-induced endotoxemia. Ten BALB/c mice per time point were injected i.p. with 100 μg of LPS and either PBS (●) or PBS containing 10 μg of rmIL-13 (○). Serum was obtained, and cytokine levels were measured at 0, 1, 2, 3, and 4 h for TNF-α and at 0, 1, 3, 5, 7, 9, and 11 h for IFN-γ and IL-12 p40. The values shown represent the mean ± SEM for ELISA data from 10 mice. The p values for statistical significance (*** indicates $p < 0.001$) are based on comparison to mice that were treated with an LD_{90} of LPS alone at time zero, and were generated using the Wilcoxon rank sum test.

11 h following LPS injection. Administration of rmIL-13 in combination with LPS resulted in a latent, but marked, suppression of LPS-induced IL-10 mRNA transcripts beginning 9 h after LPS injection, a finding consistent with the observation that IL-13 can inhibit IL-10 production in vitro (32). Together with previous in vitro data (32, 33), these data lend support to the premise that IL-13 and IL-10 function independently to effect suppression of proinflammatory cytokine production in response to LPS-induced endotoxemia. Although these findings would predict a synergistic activity for IL-10 and IL-13 in protecting mice against the lethality associated with LPS-induced endotoxemia, our attempts to demonstrate the effect of combined IL-10 and IL-13 treatment on LPS-induced lethality showed no evidence for a synergistic protective response. Further experiments will clearly be required to dissect the complex in vivo interaction occurring between these two immunomodulatory cytokines.

Taken in the context of similar protection experiments conducted with IL-10 (26, 28, 29), the findings presented here strengthen the idea that cytokines capable of antagonizing the production of proinflammatory mediators, such as TNF-α or IFN-γ, can be effective at conferring protection against the lethality associated with elevated circulating levels of these cytokines. IL-13 and IL-10 also suppress the inflammatory response by down-modulating IL-1 and nitric oxide production while up-regulating the production of IL-1Ra (3), and although not addressed in either of these systems, these factors may contribute to the protective properties of these cytokines. Clearly, the potent anti-inflammatory properties of IL-10 and IL-13 make these cytokines attractive candidates for the treatment of septic shock; however, the mechanisms of action of these two molecules within the immune system are very different. While IL-10 is recognized as a potent and general inhibitor of IFN-γ production by T and NK cells and of IL-12 production by accessory cells, the effect of IL-13 on production of these inflammatory mediators is more complex. Certain in vitro data (32, 33, 45) indicate that IL-13 can inhibit IL-12 production by LPS-activated monocytes/macrophages, and the data presented here demonstrate that IL-13 can inhibit TNF-α, IFN-γ, and IL-12 production during LPS-induced lethal endotoxemia in vivo. However, in vitro data in other systems have shown that IL-13 can act either directly or synergistically with IL-2 to induce IFN-γ production from large granular lymphocytes (31). Furthermore, D'Andrea et al. (45) have shown that in the absence of IL-10-derived signals, IL-13 can actually prime human PBMC for enhanced IL-12 and TNF-α production in response to LPS. Our data

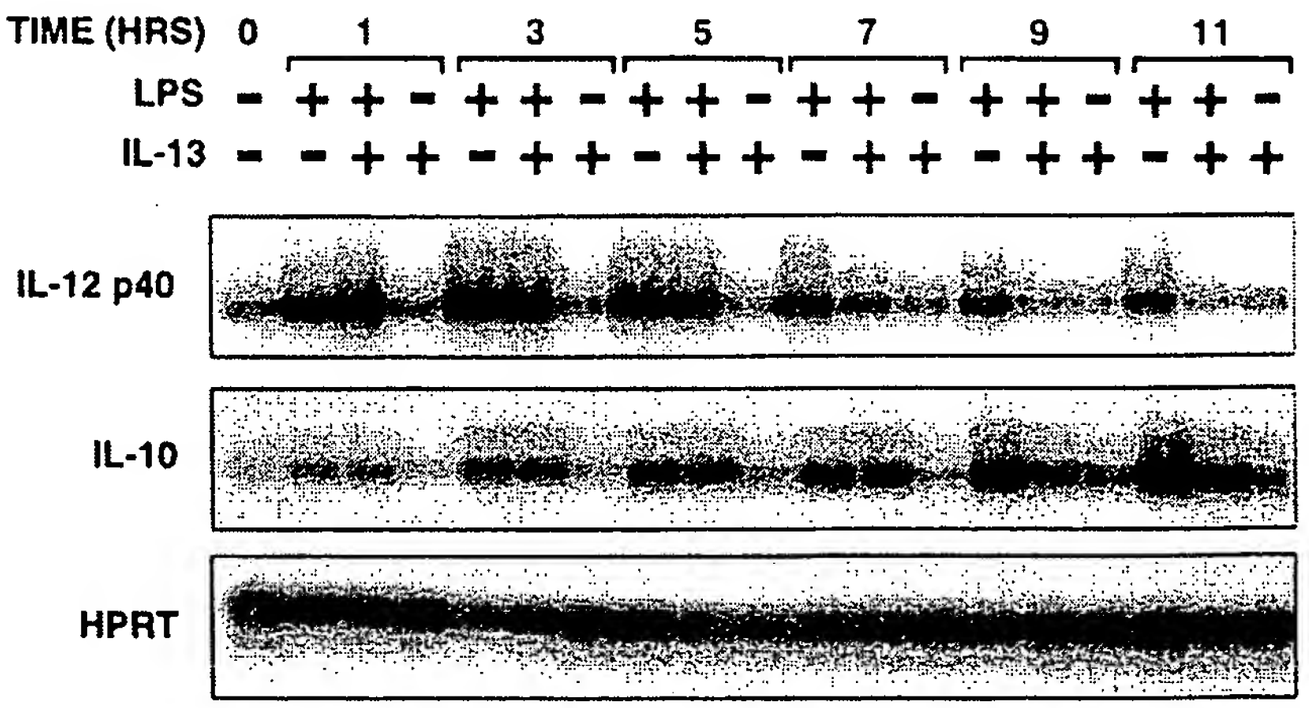

FIGURE 5. rmIL-13 inhibits the LPS-induced accumulation of IL-12 p40 and IL-10 mRNA transcripts during lethal endotoxemia. BALB/c mice were injected i.p. with 100 μg of LPS and either PBS or PBS containing 10 μg of rmIL-13, or with 10 μg of rmIL-13 alone. Groups of three mice were sacrificed at 0, 1, 3, 5, 7, 9, and 11 h, and total RNA was extracted from the pooled spleens as described in *Materials and Methods*. Total RNA (1 μg) was analyzed by reverse transcriptase-PCR analysis and DNA blotting as described in *Materials and Methods*. The immobilized DNA was hybridized to ^{32}P-labeled mouse cDNA probes specific for IL-12 p40, IL-12 p35 (data not shown), IL-10, and HPRT. The data shown are representative of results obtained in three independent RNA analysis experiments (IL-13-alone group analyzed in only one of the three experiments).

showing that rmIL-13 enhanced LPS-induced splenocyte IL-12 p40 mRNA levels at early time points following LPS administration, when the induction of IL-10 by LPS was modest, would lend some support for this role of IL-13. Based on these latter properties of IL-13 it has been suggested that in the absence of an ongoing Th2 response, IL-13 is likely to enhance APC and general phagocytic function and favor a Th1 type of immune response (45). On the other hand, acute regulation of the immune system by IL-13 could possibly be reflected in an up-regulation of B cell effector function, thus favoring a Th2 type of immune response. Determination of which of these alternatives prevails in an in vivo immune response will require careful experimentation to resolve. Thus, while showing promise as a candidate for the treatment of septic shock, the efficacy of IL-13 treatment may be dependent on the broader effects of this cytokine within the immune system.

Acknowledgments

The authors thank Dr. John Abrams (DNAX) for assistance with statistical analyses and Mary Weiss for technical support in the production of rmIL-13.

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